The value of comparative animal research: Krogh's principle facilitates scientific discoveries.

Tyler J. Stevenson*[1], Beau A. Alward[2], Francis J.P. Ebling[3], Russell D. Fernald[2], Aubrey Kelly[4], Alexander G. Ophir[4].

School of Biological Sciences, University of Aberdeen, Aberdeen United Kingdom[1]; Department of Biology, Stanford University, Stanford United States[2]; School of Life Sciences, University of Nottingham, Nottingham United Kingdom[3]; Department of Psychology, Cornell University, Ithaca United States[4].

*Corresponding Author Address:
School of Biological Sciences
University of Aberdeen
Aberdeen, Scotland
AB24 2TZ

Abstract

Biomedical research is dominated by relatively few animal models. Research has over-relied on these models due to their well-described genomes, genomic manipulations and short generation times. However, recent advances in large scale molecular sequencing experiments have revealed, in some cases, the limited similarities in experimental outcomes observed in common rodents (i.e. mice) compared to humans. The value of more varied comparative animal models includes examples such as long-term body weight regulation in seasonally breeding hamsters as a means to help understand the obesity epidemic, vocal learning in songbirds to illuminate language acquisition and maintenance, and reproduction in cichlid fish to discover novel genes conserved in humans. Studying brain peptides in prairie voles and cichlids advanced knowledge about social behavior. Taken together, experiments on diverse animal species highlight non-traditional systems for advancing our understanding of human health and well-being.

Introduction

During Charles Darwin's travels on the Beagle, he conversed with a Spanish lawyer and a German naturalist, Renous. Renous asked the Spanish lawyer '*what he thought of the King of England sending out a collector to their country, to pick up lizards and beetles, and to break stones?* To which the Spanish lawyer replied '*No man is so rich as to send out people to pick up such rubbish*' (Darwin, 1839). Investment in basic research has a long and distinguished tradition, and we are fortunate that governments continue to provide the essential financial support. Every year, nations throughout the world invest substantial amounts into basic and biomedical research with an aim of generating long-term translational outcomes that will benefit humankind. From 2000-2014, research spending adjusted for purchasing power has increased approximately 64% in the United States (~$290 to $450 billion), 57% in the European Union overall (~$200 to $350 billion) and 66% in the United Kingdom (~$20 to $30 billion Research Council United Kingdom) (van Noorden, 2016). One of the key indicators of successful returns on research investment is the number of scientific publications. As evidenced by the MEDLINE database, the United States, European Union and the United Kingdom RUK contribute to approximately 32%, 26% and 8% of the world's scientific advancement through publications, respectively (van Noorden, 2016). These patterns highlight a healthy balance between government expenditure and major scientific advances that in turn facilitate innovative technologies and knowledge that benefits human health and wellbeing.

The majority of biomedical research seeks to enhance knowledge at basic mechanistic and applied translational levels to better inform healthy and pathological conditions in humans. However, our methods under-use animal models to understand health and disease and to develop medicines. Most animals in biomedical research are mice and rats. Comparing the

proportion of human publications in 2016 for a range of animal models shows that publications using mice accounted for approximately 25% compared to human research. The summed percentage of the non-traditional animals accounted for less than 2%, illustrating a massively disproportionate amount of research funding on mice models. Yet, 'non-traditional' animal models— hamsters, songbirds, voles, and cichlid fish—have aided discoveries. These non-traditional biomedical species offer specialized, adapted genomic, physiological, immunological and behavioral traits. Comparing these traits across animals,helps identify the common underlying causes of many human conditions (see also Remage-Healey et al., 2017). Here we provide novel evidence to show that comparative animal studies yield major scientific advancements.

Biomedical models for scientific research

Granted the exponential increase in publications using 'human models' from 1968 to 2016 in PUBMED, the two most common biomedical research animal models include mice and rats. The ability to generate genetically modified mice in the 1990s ushered in a new era of research that identified the functional significance of specific genes. Consider the traditionally female hormone estogen: generating mice with a deleted estrogen-receptor gene illustrated how estrogen controls reproductive health in both females *and* males (Rissman et al., 1997). Subsequently, the capability to delete specific genes selectively in mice has increased the rate of using this model species.

However, mice are a limited model species to understand human function, so some findings do not translate well to human research, particularly in immunology (Bolker, 2012; de Souze, 2013; Drake, 2013). The comparative approach—going beyond mice, rats, and human—

can identify commonalities and differences across organisms. Unfortunately, scientific funding is substantially lower for non-traditional animal species.

Krogh's Principle and the comparative approach for biomedical animal models

August Krogh was a Danish physiologist awarded the Nobel Prize in Physiology or Medicine in 1920 for his discovery of the circulatory systems' ability to carry oxygen to muscles. In addition, Krogh proposed that '*For a large number of [scientific] problems there will be some animal of choice or a few such animals on which it can be most conveniently studied … we must apply to the zoologists to find them*' (Krogh, 1929). As noted, mice have contributed to the major scientific advancements in the last twenty years. However, as Krogh advised, many human conditions—physiological, immunological, neural, and behavioral— are best studied in an alternative model. Despite the comparative approach's advantages to understand fundamental biomedical science or many pathological or disease conditions, research continues to over-rely on mice (Beach, 1950). Substantial genomic developments such as genome-sequencing (Koboldt et al., 2013) and genome-editing (Lee et al., 2016) now allow respectively sequencing whole genomes in a matter of days and conducting precise genomic manipulations. These two tools permit genomic analyses in a range of mammals, birds, and fish.

Below, four representative animal species illustrate physiology, immunology or behaviors different from other common biomedical models (i.e. mice) but facilitate our understanding of the human condition using Krogh's principle. The comparative perspective below generates fundamental biological knowledge that can complement other biomedical models to provide a comprehensive understanding for human health and disease conditions.

Brain and hormonal control of long-term body weight regulation in hamsters.

The World Obesity Federation estimates the prevalence of obesity is approximately 35-40% of the United States population. Healthy body weight regulation involves a complex interplay between behavioral (i.e. diet), physiological, and neural pathways (Yeo & Heisler, 2012). Most research has investigated short-term regulation, balancing food intake and energy expenditure. Specific neuropeptides (e.g. agouti-related peptide, neuropeptide Y) signal energetic states: either low (undernourished) or high (over-fed) body-weight conditions. These systems determine short-term timing of meal intervals and compensatory responses to acute energy insufficiency, so genetic manipulation of such pathways often produces a clear phenotype. However, problems of healthy body weight maintenance extend beyond this well-defined neural system. Long-term hypothalamic mechanisms help regulate body weight (Ebling, 2015).

Seasonal animals provide a valuable opportunity to examine naturally occurring genomic, physiological, and behavioral changes with major translational implications for humans (Ebling, 2014; Morgan et al., 2006; Stevenson et al., 2015). For example, the Siberian hamster (*Phodopus sungorus*) has been a valuable model to study long-term changes in physiological systems, such as losing body weight (Stevenson & Prendergast, 2013) and immune function (Stevenson et al., 2014). Hamsters will show a reliable, robust, and repeatable, cycle in body weight. In the laboratory, long days similar to the summer maintain 'obese' hamsters. A simple change in the amount of light especially those that mimic short-winter days induces roughly 30% weight loss (Stevenson & Prendergast, 2015). The decrease in body weight represents a long-term change in homeostatic control of energy balance. Unfortunately, the common mouse models have lost the photoperiodic change in body weight and are therefore, not suitable for examining long-term body weight regulation. In the hamster brain, there is a discrete population of cells referred to as

tanycytes that are localized along the 3rd ventricle in the brain. These cells have been proposed to control the long-term changes in hamster body weight (Lewis and Ebling, 2017). In mice, tanycytes have been shown to be critical for the detection of plasma glucose, a strong measure of energetic state (Orellana et al. 2012). The prevailing idea is that the metabolic state of the animal is detected by tanycytes (Bolborea and Dale 2013), and then control body weight through a well-defined neuropeptide circuit (Yeo & Heisler, 2012). However, experiments conducted in mice predominantly examine the acute, short term impacts of diet on body weight regulation. The major advantage of the hamster model is the demonstration that long-term neuro-morphological changes by tanycytes that in turn govern key brain regions involved in energy balance. The current evidence indicates that the locally produced thyroid hormone, triiodothyronine, in tanycytes controls long-term changes in body weight (Murphy et al., 2012). How the environment and diet impacts the ability of tanycytes to produce triiodothyronine and the subsequent impact on the short-term brain circuits is poorly understood. A greater understanding of the role of thyroid hormone action in the hamster tanycytes will better inform how obesity is maintained over longer time scales in humans.

Songbirds enhance our understanding of innately programmed learned behavior: language.

The National Institute on Deafness and Other Communication Disorders reports 6 to 8 million people in the United States have language impairments (NIDCD, 2016). These include: stutters, spasmodic dysphonia and autism. The majority of disorders develop during childhood or adolescence and show remarkable sex-biases (Bale et al., 2010). Research into the underlying genetic, molecular, and neural control of language is hindered by the lack of animals that learn the species vocalizations. Songbirds, and in particular the zebra finch, represent the model

system that has led to insights about the neurobiological control of learned vocalizations. A series of elegant experiments during the 1980s-1990s demonstrated that discrete brain regions are necessary for songbirds to learn their songs (Fee & Scharff, 2010; reviewed in Alvarez-Buylla et al., 1992). The two main brain regions are essential for producing songbird vocalization (hyperpallium, i.e. HVC, and caudal medial pallium; Bolhuis & Moorman, 2015). These two regions have functional analogy to areas in the human brain implicated in language (Broca's and Wernicke's areas), especially acquisition of language in infants and maintenance in adulthood (Bolhuis & Moorman, 2015).

Many songbirds, including the zebra finch, show a substantial neurogenesis in the adult brain, that is, new neurons born and recruited to HVC (Alvarez-Buylla et al., 1992; Alward et al., 2014). Locally produced hormones in the pallium (e.g. HVC) improve the perception of the species' own song (Remage-Healey et al., 2013) and improve the birds' ability to produce a high quality song (Alward et al., 2013, 2016; Rouse et al., 2015). The technological development of high-throughput analyses (such as microarray assays) has permitted the identification of large changes in gene expression in the songbird brain that are likely involved in vocal quality, a feature similar to how well humans produce speech (Replogle et al., 2008). These brain-derived hormones are likely regulating gene expression in brain regions involved in songbird vocalizations (Stevenson et al., 2012a). How the brain generates these new neurons, how the new neurons are recruited into HVC, and what triggers the functional outcome of these new neurons for songbird vocalizations remains unsolved. A deeper knowledge about these basic questions would aid treatment of language disorders in humans.

Elucidating the neural mechanisms of social behavior using the prairie vole

In the past few decades, prairie voles have become a valuable animal for understanding the complex brain networks that control social behavior (Insel & Shapiro, 1992; McGraw & Young, 2010). Prairie voles have received substantial attention, largely based on (1) a rich literature on their natural history and behavioral ecology, (2) leveraging tools developed in classic rodent models, due to their close genetic relationship, and (3) rare but defining behaviors shared with humans but absent in common biomedical models (e.g. mice), such as communal living, social monogamy, and biparental care (Carter, 1998; Lukas & Clutton-Brock, 2012; McGraw & Young, 2010). In this respect, prairie voles illustrate the Krogh Principle: for this species, general knowledge bridging behavioral ecology and neurobiology can expand. Moreover, this species is well suited to serve as a model for several aspects of human social behavior and dysfunction (Carter 2007; Young 2001).

By far, the most commonly studied form of social behavior in prairie voles is pair bonding. Comparative studies in the 1990s made the remarkable discovery that the neuropeptides, vasopressin and oxytocin, are critical for monogamous relationships (Carter et al. 1995). These peptides were identified in numerous brain regions now known as critical for social behavior and characterized as a 'pair bonding neural circuit' (Carter et al., 1997; Insel & Young, 2001; Young &Wang, 2004). Recent technological advances in molecular biology (e.g., epigenetic and optogenetic techniques) have expanded our understanding of the neural circuitry of reproductive decisions in prairie voles in a manner unavailable in common biomedical models (Amadei et al., 2017).

The value of prairie voles extends well beyond the study of pair bonding, and this species offers opportunities to address other pressing issues in behavioral neuroscience and general biology. For example, voles have been valuable to study biparental care (Kelly et al. 2017; Bales

et al., 2007, Wang and Novak, 1994; Prounis et al., 2015; Hammock, 2015), aggression (Gobrogge and Wang 2011), social recognition (Blocker & Ophir 2015; Zheng et al., 2013), neurogenomics of sociosexual behavior (McGraw et al., 2012), non-sexual relationships (Beery & Zucker, 2010), emotional regulation of the cardiovascular system and mind-heart interactions (Grippo et al., 2012), fMRI analysis of brain activity in awake animals (Yee et al., 2016), and reward and addiction (Aragona et al. 2007; Ryabinin & Hostetler, 2016). Recently, prairie voles have also emerged as a promising system for studies that integrate the role of cognition (i.e., learning and memory) in mating systems to provide a more comprehensive understanding of the suite of factors that drive reproductive strategies and social-decision making (Ophir et al. 2008; Ophir 2017; Rice et al. 2017; Okhovat et al. 2015; Phelps and Ophir 2009). Thus, prairie voles represent a species with numerous uses in basic and translational research. With a fully sequenced genome (McGraw & Young, 2010) and an established network of researchers interested in understanding its behavioral ecology, development, neurobiology, and molecular genetics, prairie voles hold extraordinary potential to address questions focused on the neural and genetic basis of social behavior.

Cichlid fish species facilitate neurobiological discoveries of plasticity in social behavior

Behavior shaped by natural selection overwhelmingly results from selective pressures on social interactions. For example, group-living animals, including humans, form dominance hierarchies with obligatory social interactions. Nevertheless, most animal experiments are done on single individuals, typically in asocial, non-natural conditions. This is partly because keeping rat and mouse model colonies and observing them during their normal nocturnal activities is hard, expensive, and impractical.

In contrast, fish offer unusual opportunities for understanding social behavior, its mechanistic underpinnings, and its role in reproduction. Cichlid fish that have evolved in the African rift lakes comprise more than 2000 species, evolving a broad range of social systems including female, male, or bi-parental care; monogamous pairs with helpers; and polygamous harems with helpers (Awata et al., 2005). Because cichlids offer experimental access at several levels of biological organization, their analysis has uncovered many widely conserved neural, physiological, and molecular mechanisms (White et al., 1998; Fernald, 2006; Ma et al., 2015; Maruska and Fernald, 2014).

Here we describe research on a well-studied African cichlid fish, *Astatotilapia burtoni*, from Lake Tanganyika, east Africa. *A. burtoni* males in nature congregate around food sources and are either dominant or non-dominant. Dominant males actively defend territories, court, and reproduce with females, while non-dominant males look like the females that they mimic, do not reproduce, and school together (Fernald & Hirata, 1977a). Crucially, these naturally occurring and reversible behavioral phenotypes can easily be observed in the laboratory, allowing experiments designed to answer questions at multiple biological levels.

Gonadotropin-releasing hormone (GnRH) is essential for regulating reproduction (Fernald & White, 1999; Stevenson et al., 2012b). Release of GnRH from the brain's hypothalamus controls the pituitary's production of gonadotropins responsible for gonadal (sex organ) development in all vertebrates; this process has been highly conserved during 500 million years of vertebrate evolution. Using *A. burtoni* allowed the first cloning of the gene controlling this peptide in non-mammalian vertebrates (White et al., 1995), revealing not one but three genes. This discovery in fish then facilitated the identification of a second GnRH gene in humans (White et al., 1998) and other mammals (Kasten et al., 1996). What is particularly striking in this

fish species are the observations that 1) GnRH containing neurons increase 8X in volume when an animal becomes dominant (Francis et al., 1993), 2) GnRH neurons are interconnected and fire synchronously in response to social ascent (Ma et al., 2015), 3) the GnRH production is regulated by social status (Soma et al., 1996), and 4) that males reared with adults show delayed maturation relative to those reared without adults (Davis & Fernald, 1990). The availability of new genetic techniques has enhanced the roles of non-traditional fish model systems for discoveries with direct relevance to human health and wellness (Juntti et al., 2013).

An individual's social status is important because the amount of GnRH in key brain regions is associated with the dominance rank, which is typically established via physical fights. Dominance is maintained through social signals including postures that demonstrate size or show teeth (Huntingford & Turner, 1987). In *A. burtoni*, non-dominant males attend closely to dominant males, demonstrating that they anticipate movements of dominant males (Desjardins et al., 2012). But what do these animals know about their environment? Is it possible for cichlids to recognize the relative strength of other fish? In the field, colonies of *A. burtoni* range in size from a few dozen to hundreds (Fernald & Hirata, 1977) meaning that they could use a strategy of fighting with every male in the colony to identify one they could beat. However, by watching other male-male interactions, cichlids can infer their chances of winning a fight by viewing (i.e. 'by-stander') pairwise fights of other fish (Grosenick et al., 2007). This skill, known as transitive inference, is a form of deductive reasoning that allows inference of a relationship among items that have not been explicitly compared. Piaget (1928) described this as a key milestone in the development of human infants older than 3 years, and it has also been described for non-human primates (Rapp et al., 1996), rats (Roberts & Phelps, 1994) and birds (Bond et al., 2003).

Social information causes profound genomic, neural, physiological, and behavioral responses in *A. burtoni* (reviewed in Fernald, 2012; Maruska, 2015). When non-dominant males have an opportunity to ascend in social rank, expression of the immediate early gene *egr-1* is upregulated within the POA, a critical hub within the social behavior network that mediates adaptive behavioral responses. Moreover, expression of gonadotropins and their receptors are higher in dominant males and increased in ascending males (Maruska et al., 2011). Within 30 minutes of social opportunity, expression levels of sex steroid hormones and their cognate receptors in the brain and gonads also increase (Maruska et al., 2013), as ascending males begin showing high levels of aggressive and reproductive behavior during social opportunity.

Many causal molecular mechanisms of *A. burtoni* behavior are unknown, but advances in genetic techniques have the potential to reveal new discoveries. For instance, recent work with gene editing technology (CRISPR-Cas9) identified a critical role of a receptor for one hormone (prostaglandin $F_{2\alpha}$) in regulating reproductive behavior in female *A. burtoni* (Juntti et al., 2016). New techniques have started a new era of animal research, allowing social behavior scientists to use diverse social systems present among many different taxa to understand the evolutionary trajectories of social behavior.

Conclusions and future directions

This paper highlights the comparative approach in animal experimentation to better understand human health and diseases. Common biomedical models, particularly mice and rats, are well-established systems that have made significant gains in basic and translational research for the benefit of human health and wellness. However, in some cases highlighted here, other specialized physiological responses and social behaviors produced in non-traditional animal models (i.e. hamsters, songbirds, voles, and cichlids) are more suited for scientific investigation.

Given the remarkable technological advances, such as whole genome sequencing and genome editing, the tools that were once the sole realm of mouse models can be readily applied to a range of animals. To successfully apply Krogh's principle, large-scale funders need to incorporate strategic priorities that focus on the scientific gains afforded by the comparative approach.

Acknowledgements

There are no conflicts of interest to declare. This paper developed from the 2016 Early Career Impact Award from the Federation of Associations in Behavioral & Brain Sciences to TJS. TJS has received funding from The Leverhulme Trust. FJPE is in receipt of funding from the BBSRC (BB/M001555/1). The National Institutes of Health has funded RDF (NS 034950, NS093277, NIMH 087930), AGO (HD079573, IOS-1354760) and AMK (HD081959). BAA is an Arnold O. Beckman postdoctoral fellow.

References

Alvarez-Buylla, A., Ling, C.Y., & Nottebohm, F. (1992) High vocal center growth and its relation to neurogenesis, neuronal replacement and song acquisition in juvenile canaries. *J. Neurobiol. 23,* 396-406.

Alward, B.A., Madison, F.N., Parker, S.E., Balthazart, J. & Ball, G.F., (2016). Pleiotropic control by testosterone of a learned vocal behavior and its underlying neuroplasticity. *eNeuro 3,* (1).

Alward, B.A., Mayes, W.D., Peng, K., Stevenson, T.J., Balthazart, J., & Ball, G.F. (2015). Dissociable effects of social context on song and doublecortin immunoreactivity in male canaries. *Eur J Neurosci. 40,* 2941-2947.

Alward, B.A., Balthazart, J., & Ball, G.F. (2013) Differential effects of global versus local testosterone on singing behavior and its underlying neural substrate. *Proc Natl Acad USA 110,* 19573-19578.

Amadei, E.A., Johnson, Z.V., Jun Kwon, Y., Shpiner, A.C., Saravanan, V., Mays, W.D., Ryan, S.J., Walum, H., Rainnie, D.G., Young, L.J., Liu, R.C. (2017). Dynamic corticostriatal activity biases social bonding in monogamous female prairie voles. *Nature 546,* 297-301.

Aragona, B.J., Detwiler, J.M., & Wang, Z. (2007). Amphetamine reward in the monogamous prairie vole. *Neurosci Lett 418,* 190-194.

Awata, S., Munehara, H., & Kohda, M. (2005). Social system and reproduction of helpers in a cooperatively breeding cichlid fish (*Julidochromis ornatus*) in Lake Tanganyika: field observations and parentage analyses. *Behav Ecol Sociobiol. 58,* 506-516.

Bale, T.L., Baram, T.Z., Brown, A.S., Goldstein, J.M., Insel, T.R., McCarty, M.M., Nemeroff, C.B., Reyes, T.M., Simerly, R.B., Susser, E.S., & Nestler, E.J., (2010). Early life programming and neurodevelopmental disorders. *Biol. Psychiatry 68,* 314-319.

Bales, K.L., Plotsky, P.M., Young, L.J., Lim, M.M., Grotte, N., Ferrer, E., & Carter, C.S. (2007). Neonatal oxytocin manipulations have long-lasting, sexually dimorphic effects on vasopressin receptors. *Neuroscience 144,* 38-45.

Beach, F.A., (1950). The snark was a boojum. *American Psychologist 5,* 115-124.

Beery, A.K., & Zucker, I. (2010). Oxytocin and same-sex social behavior in female meadow voles. *Neuroscience 169,* 665-673.

Blocker, T.D. & Ophir, A.G. (2015). Social recognition in paired but not single male prairie voles. *Anim Behav 108,* 1-8.

Bolborea, M., & Dale, N., (2013) Hypothalamic tanycytes: potential roles in the control of feeding and energy balance. *Trends Neurosci. 36*, 91-100.

Bolhuis, J.J., & Moorman, S., (2015). Birdsong memory and the brain: in search of the template. *Neurosci Behav. Rev. 50*, 41-55.

Bolker, J. (2012). Model organisms: There's more to life than rats and flies. *Nature. 491*, 31–33.

Bond, A.B., Kamil, A.C. & Balda, R.P. (2003). Social complexity and transitive inference in corvids. *Anim. Behav. 65*, 479-487.

Carter, C.S. (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinol 23*, 779-818

Carter, C.S. (2007). Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders? *Behav Brain Res 176*, 170-186.

Carter, C.S., DeVries, A.C., & Getz, L.L. (1995). Physiological substrates of mammalian monogamy: the prairie vole model. *Neurosci Biobehav Rev 19*, 303-314.

Carter, C.S., DeVries, A.C., Taymans, S.E., Roberts, R.L., Williams, J.R., & Getz, L.L. (1997). Peptides, steroids, and pair bonding. *Ann N Y Acad Sci 807*, 260-272

Darwin, C., (1839). The voyage of the Beagle. Pg 130.

Davis, M.R. & Fernald R.D. (1990). Social control of neuronal soma size. *J. Neurobiol. 21*, 1180-1188.

Desjardins, J.K., Hofmann, H.A. & Fernald, R.D. (2012). Social context influences aggressive and courtship behavior in a cichlid fish. *PLoS ONE 7*, e32781.

de Souza, N. (2013). Model organisms: Mouse models challenged. *Nat Methods 10*, 288.

Drake, A.C. (2013). Of mice and men: what rodent models don't tell us. *Cell Mol Immunol.* 10, 284–285.

Ebling, F.J. (2014). On the value of seasonal mammals for identifying mechanisms underlying the control of food intake and body weight. *Horm. Behav. 66*, 56-65.

Ebling, F.J. (2015). Hypothalamic control of seasonal changes in food intake and body weight. *Front Neuroendocrinol. 37,* 97-107.

Fee, M.S., & Scharff, C. (2010). The songbird as a model for the generation and learning of complex sequential behaviors. *ILAR J 51,* 362-377.

Fernald, R.D. (2006). Casting a genetic light on the evolution of eyes. *Science 313,* 1914-1918.

Fernald, R.D., (2012) Social control of the brain. *Annu Rev Neurosci. 35*, 135–151.

Fernald, R.D. & Hirata N. R. (1977) Field study of *Haplochromis burtoni*: Habitats and co-habitants. *Environ. Biol. Fishes. 2,* 299-308.

Fernald, R.D., & White, R.B. (1999). Gonadotropin-releasing hormone genes: phylogeny, structure and functions. *Front. Neuroendocrinol.* 20, 224-240.

Francis, R. C., Soma, K., & Fernald R.D. (1993). Social regulation of the brainpituitary-gonadal axis. *Proc. Natl Acad. Sci. 90,* 7794-7798.

Gobrogge, K.L. & Wang, Z.W. (2011). Genetics of aggression in voles. *Adv Genet 75,* 121-150.

Grippo, A.J., Moffitt, J.A., Sgoifo, A., Jepson, A.J., Bates, S.L., Chandler, D.L., McNeal, N., Preihs, K. (2012). The integration of depressive behaviors and cardiac dysfunction during an operational measure of depression: investigating the role of negative social experiences in an animal model. *Psychosom Med 74,* 612-619.

Grosenick, L., Clement, T.S. & Fernald, R.D. (2007). Fish can infer social rank by observation alone. *Nature 445*, 429-432.

Hammock, E.A. (2015). Developmental perspectives on oxytocin and vasopressin. *Neuropsychopharmacology 40,* 24-42.

Huntingford, F.A. & Turner, A.K. (1987). Animal Conflict. London; New York: Chapman and Hall.

Insel, T.R., & Shapiro, L.E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc Natl Acad Sci USA 89,* 5981-5985

Insel, T.R., & Young, L.J. (2001). The neurobiology of attachment. *Nat Rev Neurosci 2,* 129-136.

Juntti, S.A., Hu C.K., & Fernald, R.D. (2013). Tol2-mediated generation of a transgenic haplochromine cichlid, *Astatotilapia burtoni PLoS ONE 8(10),* e77647

Kelly, A.M., Hiura, L.C., Saunders, A.G., & Ophir, A.G. (2017). Oxytocin neurons exhibit extensive functional plasticity due to offspring age in mothers and fathers. *Integr Comp Biol In Press*

Koboldt, D.C., Steinberg, K.M., Larson, D.E., Wilson, R.K., & Mardis, E.R. (2013) The next-generation sequencing revolution and its impact on genomics. *Cell 155,* 27-38.

Krogh, A., (1929). The Progress of Physiology. *Amer. J. Physiol. 90,* 243-251.

Lee, H.B., Sundberg, B.N., Sigafoos, A.N., & Clark, K.J. (2016). Genome engineering with TALEN and CRISPR systems in neuroscience. *Front. Genet. 7,* 47.

Lewis, J.E., & Ebling, F.J.P., (2017). Tanycytes as regulators of seasonal cycles in neuroendocrine function. *Front. Neurol. 8,* 79.

Lukas, D., & Clutton-Brock, T. (2012). Cooperative breeding and monogamy in mammalian societies. *Proc Biol Sci 279,* 2151-2156.

Ma, Y., Juntti, S.A., Hu, C.K., Huguenard, J.R., & Fernald R.D. (2015). Electrical synapses connect a network of gonadotropin releasing hormone neurons in a cichlid fish. *Proc Natl Acad Sci 112,* 3805-3810.

403 Maruska, K.P., (2015) Social transitions cause rapid behavioral and neuroendocrine changes.
404 *Integr Comp Biol*. *55*, 1–13.
405 Maruska, K.P., Levavi-Sivan, B., Biran, J., & Fernald, R.D., (2011) Plasticity of the reproductive
406 axis caused by social status change in an African cichlid fish: I. Pituitary gonadotropins.
407 *Endocrinology*. *152*, 281–290.
408 Maruska, K.P. & Fernald R.D. (2014) Social regulation of gene expression in the African cichlid
409 fish *Astatotilapia burtoni* Oxford Handbook of Psychology, pp 52-78.
410 Maruska, K.P., Zhang, A., Neboori, A., Fernald, R.D., (2013) Social opportunity causes rapid
411 transcriptional changes in the social behaviour network of the brain in an African cichlid
412 fish. *J Neuroendocrinol*. *25*, 145–157.
413 McGraw, L.A., & Young, L.J. (2010). The prairie vole: an emerging model organism for
414 understanding the social brain. *Trends Neurosci 33*, 103-109.
415 McGraw, L.A., Davis, J.K., Thomas, P.J., Program, N.C.S., Young, L.J., & Thomas, J.W.
416 (2012). BAC-based sequencing of behaviorally-relevant genes in the prairie vole. *PLoS*
417 *One 7*, e29345.
418 Morgan, P.J. Ross, A.W. Mercer, J.G. & Barrett, P. (2006). What can we learn from seasonal
419 animals about the regulation of energy balance? *Prog. Brain Res. 153*, 325-337.
420 Murphy, M., Jethwa, P.H., Warner, A., Barrett, P., Nilaweera, K.N., Brameld, J.M., & Ebling,
421 F.J., (2012) Effects of manipulating hypothalamic triiodothyronine concentrations on
422 seasonal body weight and torpor cycles in Siberian hamsters. *Endocrinol. 153*, 101-112.
423 National Institute on Deafness and Other Communication Disorders (NIDCD). (2106).
424 https://www.nidcd.nih.gov/health/statistics/statistics-voice-speech-and-language.

425 Okhovat, M., Berrio, A., Wallace, G., Ophir, A.G., & Phelps, S.M. (2015). Sexual fidelity trade-
426 offs promote regulatory variation in the prairie vole brain. *Science 350,* 1371-1374.
427 Ophir, A.G., Wolff, J.O., & Phelps, S.M. (2008). Variation in neural V1aR predicts sexual
428 fidelity and space use among male prairie voles in semi-natural settings. *Proc Natl Acad*
429 *Sci USA 105,* 1249-1254.
430 Ophir, A.G. (2017). Navigating Monogamy: Nonapeptide Sensitivity in a Memory Neural
431 Circuit May Shape Social Behavior and Mating Decisions. *Front Neurosci 11,* 397.
432 Orellana, J.A., Saez, P.J., Cortes-Campos, C., Elizondo, R.J., Shoji, K.F., Contreras-Duarte, S.,
433 Figueroa, V., Velarde, V., Jiang, J.X., Nualart, F., Saez, J.C., & Garcia, M.A., (2012).
434 Glucose increases intracellular free Ca2+ in tanycytes via ATP released through connexin
435 43 hemichannels. *Glia 60,* 53-68.
436 Phelps, S.M., & Ophir, A.G. (2009). Monogamous brains and alternative tactics: Neuronal
437 V1aR, space use and sexual infidelity among male prairie voles. Cognitive Ecology II (*eds:*
438 *Dukas R, Ratcliffe JM*) Chicago: University of Chicago Press.
439 Piaget, J. (1928). Judgement and Reasoning in the Child. London: Kegan, Paul, Trench &
440 Trubner.
441 Prounis, G.S., Foley, L., Rehman, A., & Ophir, A.G. (2015). Perinatal and juvenile social
442 environments interact to shape cognitive behaviour and neural phenotype in prairie voles.
443 *Proc Biol Sci 282,* 1819.
444 Rapp, P.R., Kansky, M.T. & Eichenbaum, H. (1996). Learning and memory for hierarchical
445 relationships in the monkey: effects of aging. *Behav. Neurosci. 110,* 887-897.

Remage-Healey, L., Jeon, S.D., & Joshi, N.R. (2013) Recent evidence for rapid synthesis and action of oestrogens during auditory processing in a songbird. *J Neuroendocrinol. 25,* 1024-1031.

Remage-Healey, L., Krentzel, A.A., Macedo-Lima, M., & Vahaba, D., (2017). Species diversity matters in biological research. *Policy Insights Behav Brain Sci.* 1-9.

Replogle, K., Arnold, A.P., Ball, G.F., Band, M., Bensch, S., Brenowitz, E.A., Dong, S., Drnevich, J., Ferris, M., George, J.M., Gong, G., Hasselquist, D., Hernandez, A.G., Kim, R., Lewin, H.A., Liu, L., Lovell, P.V., Mello, C.V., Naurin, S., Rodriguez-Zas, S., Thimmapuram, J., Wade, J., & Clayton, D.F. (2008). The songbird neurogenomics (SoNG) initiative: community-based tools and strategies for study of brain gene function and evolution. *BMC Genomic 9,* 131.

Rice, M.A., Hobbs, L.E., Wallace, K.J., & Ophir, A.G. (2017). Cryptic sexual dimorphism in spatial memory and hippocampal oxytocin receptors in prairie voles (*Microtus ochrogaster*). *Horm Behav 95,* 94-102.

Rissman, E.F., Wersinger, S.R., Taylor, J.A., & Lubahn, D.B., (1997). Estrogen receptor function as revealed by knockout studies: neuroendocrine and behavioral aspects. *Horm. Behav. 31,* 232:243.

Roberts, W.A. & Phelps, M.T. (1994). Transitive inference in rats: a test of the spatial coding hypothesis. *Psychol. Sci. 5,* 368-374.

Rouse, M.L. Stevenson, T.J., Fortune, E.S., & Ball, G.F. (2015) Reproductive state modulates testosterone-induced singing in adult female European starlings. *Horm. Behav. 72,* 78-87.

Ryabinin, A.E., & Hostetler, C.M. (2016). Prairie Voles as a Model to Screen Medications for the Treatment of Alcoholism and Addictions. *Int Rev Neurobiol 126,* 403-421.

469 Soma, K.K., Francis, R.C., Wingfield, J.C. & Fernald R.D. (1996). Androgen regulation of
470 hypothalamic neurons containing gonadotropin-releasing hormone in a cichlid fish:
471 Integration with social cues. *Horm. Behav. 30,* 216-226.
472 Stevenson, T.J., & Prendergast, B.J. (2013). Reversible DNA methylation regulates seasonal
473 photoperiodic time measurement. *Proc. Natl. Acad. Sci. 110,* 16651-16656.
474 Stevenson, T.J., & Prendergast, B.J. (2015). Photoperiodic time measurement and seasonal
475 immunological plasticity. *Front. Neuroendocrinol. 37,* 76-88.
476 Stevenson, T.J., Replogle, K., Drnevich, J., Clayton, D.F., & Ball, G.F. (2012a). High throughput
477 analysis reveals dissociable gene expression profiles in two independent neural systems
478 involved in the regulation of social behavior. *BMC Neurosci. 13,* 126.
479 Stevenson, T.J., Hahn, T.P., MacDougall-Shackleton, S.A., & Ball, G.F. (2012b). Gonadotropin-
480 releasing hormone plasticity: a comparative perspective. *Front Neuroendocrinol. 33,* 287-
481 300.
482 Stevenson, T.J., Onishi, K.G., Bradley, S.P. & Prendergast, B.J. (2014) Cell-autonomous
483 iodothyronine deiodinase expression mediates seasonal plasticity in immune function.
484 *Brain Behav Immun 36,* 61-70.
485 Stevenson, T.J., Visser, M.E., Arnold, W., Barrett, P., Biello, S., Dawson, A., Denlinger, D.L.,
486 Dominoni, D., Ebling, F.J., Elton, S., Evans, N., Ferguson, H.M., Foster, R.G., Hau, M.,
487 Haydon, D.T., Hazlerigg, D.G., Heideman, P., Hopcraft, J.G.C., Jonsson, N.N., Kronfeld-
488 Schor, N., Kumar, V., Lincoln, G.A., MacLeod, R., Martin, S.A.M., Martinez-Bakker, M.,
489 Nelson, R.J., Reed, T., Robinson, J.E., Rock, D., Schwartz, W.J., Steffan-Dewenter, I.,
490 Tauber, E., Thackeray, S.J., Umstatter, C., Yoshimura, T., Helm, B. (2015). Disrupted
491 seasonal biology impacts health food security and ecosystems. *Proc R Soc B 282,* 1-10.

Van Noorden, R. (2016). China by the numbers. *Nature*, 534:453.

Wang, Z., & Novak, M. (1994). Alloparental care and the influence of father presence on juvenile prairie voles, *Microtus ochrogaster*. *Anim Behav 47,* 281-288.

White, S.A., Kasten, T.L., Bond, C.T., Adelman, J.P., & Fernald, R.D. (1995). Three gonadotropin-releasing hormone genes in one organism suggest novel roles for an ancient peptide. *Proc. Natl Acad. Sci. 92,* 8363-8367.

White, R.B., Eisen, J.A., Kasten, T.L., & Fernald, R.D. (1998) Second gene for gonadotropin-releasing hormone in humans. *Proc Natl Acad Sci 95,* 305-309.

Yee, J.R., Kenkel, W.M., Kulkarni, P., Moore, K., Perkeybile, A.M., Toddes, S., Amacker, J.A., Carter, C.S., & Ferris, C.F. (2016). BOLD fMRI in awake prairie voles: A platform for translational social and affective neuroscience. *NeuroImage 138,* 221-232.

Yeo, G.S. & Heisler, L.K. (2012) Unraveling the brain regulation of appetite: lessons from genetics. *Nat Neurosci 15,* 1343-1349.

Young, L.J. (2001). Oxytocin and vasopressin as candidate genes for psychiatric disorders: lessons from animal models. *Am J Med Genet 105,* 53-54.

Young, L.J., & Wang, Z. (2004). The neurobiology of pair bonding. *Nat Neurosci 7,* 1048-1054.

Zheng, D.J., Larsson, B., Phelps, S.M., Ophir, A.G. (2013). Female alternative mating tactics, reproductive success and nonapeptide receptor expression in the social decision-making network. *Behav Brain Res 246,* 139-147.

513